Centaurea corymbosa, a cliff-dwelling species tottering on the brink of extinction: A demographic and genetic study

(seed dispersal/gene flow/mating system/endemism/conservation biology)

BRUNO COLAS*†, ISABELLE OLIVIERI†‡, AND MIQUEL RIBA§

*Conservatoire Botanique National de Porquerolles, Castel Sainte Claire, rue Sainte Claire, 83418 Hyères, France; †Institut des Sciences de l'Evolution, UMR 5554, Université de Montpellier 2, Place Eugène Bataillon, 34095 Montpellier cedex 05, France and Station de Génétique et Amélioration des Plantes, Institut National de la Recherche Agronomique de Montpellier, Domaine de Melgueil, 34130 Mauguio, France; and §Centro de Recerca Ecologica i Aplicacions Forestals, Universitat Autonoma de Barcelona, Bellaterra 08193, Barcelona, Spain

ABSTRACT   *Centaurea corymbosa* (Asteraceae) is endemic to a small area (≤ 3 km^2), and <500 individuals reproduce in any given year. Nevertheless, enzyme polymorphism was found within and among the six local extant populations, the most distant at 2.3 km. Levels of gene flow among populations and seed and pollen dispersal data indicated very low dispersal capacity. Rarity of long distance dispersal events coupled with traits such as prolonged juvenile period, monocarpy, and self-incompatibility precludes the establishment of new populations and thus the evolution toward colonization ability through increased dispersal rate, polycarpy, or self-compatibility. The species thus appears to be trapped on an evolutionary dead-end toward extinction, even though, from a preliminary introduction experiment, we conclude that several nearby unoccupied sites would be suitable for the species.

The importance of dispersal processes for present trends in changes of plant distribution patterns recently has been highlighted. Declining species seem to have less dispersal mechanisms (considering dispersal in both space and time) than currently spreading species (1). Nevertheless, as pointed out by Gaston (2), although it seems likely that, in some instances, poor dispersal abilities cause rarity, in others it may well be a response to rarity, i.e., a consequence of habitat specialization (*sensu* ref. 3). Indeed, dispersal will be selected against if either the probability of reaching a suitable site or the probability of local extinction (or both) is low (4, 5), and recently, it has been shown that dispersal ability could evolve very fast (6, 7). Thus, although a rare species might have a low dispersal rate, the original reason why it is rare might actually be the lack of suitable sites rather than a low dispersal rate. Although, clearly, seed dispersal is a prerequisite for colonization, pollen dispersal after individual establishment, either from source populations or among colonists, can be limiting. The importance of pollen dispersal is highlighted by studies of flowering plants in which pollen movement is often the major component of gene flow (8–11).

Current theoretical work emphasizes the evolutionary role of both genetics and ecology in population dynamics (5, 12, 13). According to Schemske *et al.* (14), 95% of empirical studies of rare plants have taken either a genetic (35%) or an ecological (60%) approach. In this paper, and in contrast to most previous studies, we combine both genetic and demographic approaches to examine the causes of the limited distribution of a remarkably narrow endemic plant species, *Centaurea corymbosa* Pourret (Asteraceae), which occupies an area of only 3 km^2 (see below). Our study had three main objectives. First, allozyme studies were

used to assess the genetic variability of the species and to obtain an indirect measure of gene flow within and among populations. Second, field observations of extant populations were carried out to quantify within-population seed dispersal distances and the extent to which pollen dispersal may limit seed set. Third, an experimental introduction study was undertaken to test the hypothesis that habitat availability is the primary factor limiting colonization ability. Our results suggest that gene flow through either seed or pollen dispersal, if any, is highly restricted among neighboring local populations and that habitat specialization, landscape structure, and life history all preclude the evolution of either long distance dispersal or self-compatibility in *C. corymbosa*, two traits that would allow the species to spread, given that many suitable sites are readily available.

MATERIALS AND METHODS

Geographic Distribution, Habitat, and Census Size of *C. corymbosa*. *C. corymbosa* is endemic to the Massif de la Clape near Narbonne in southern France (15, 16). The Massif de la Clape is a limestone plateau of $\approx 10 \times 5$ km^2 near the Mediterranean sea (Fig. 1). The species grows on the top of the cliffs and in nearby rocky areas of open vegetation, on North-to-West exposures. It does not occur in either the pinewoods or the garrigues surrounding cliffs. It occurs on <10% of the massif (≈ 3 km^2; Fig. 1) although many sites in the remaining 90% of the massif appear suitable. Only six populations have been recorded (E1, E2, A, Cr, Pe, and Po) 0.3 to 2.3 km apart (Fig. 1). In June 1995, 494 reproducing individuals could be recorded on the whole massif (ref. 17; Fig. 1), so the effective size at the species level is expected to be very small. Total population size was estimated to be ≈ 6500 individuals, including both juveniles and adults.

C. corymbosa is a self-incompatible, monocarpic perennial. Current demographic studies show that it may live as a rosette for 3–10 years before flowering (unpublished work). Pollination is achieved mainly by small Hymenoptera. Germination rates from current year seeds were very high in good experimental conditions (75–100% at 10–20°C in moistened filter paper in Petri dish). Seeds may remain alive in the soil for more than 1 year provided the required conditions for germination are not met. In a given year, however, less than 10% of seeds may remain dormant for 1 year (unpublished data), so that dormancy is unlikely to play an essential role in *C. corymbosa* dynamics.

There is to date no phylogenetic studies within the subgenus *Acrolophus*, to which *C. corymbosa* belongs (15). We are currently studying enzyme polymorphism in *Centaurea maculosa*, a widespread species of the same subgenus, also occurring in southern France. Preliminary results (H. Fréville, unpublished data) suggest that *C. corymbosa* derive from *C. maculosa* by a founder effect following demographic bottlenecks or

Abbreviations: DIST, distance to the nearest neighbor flowering plant; DENS, number of flowering plants in a 10-m radius.
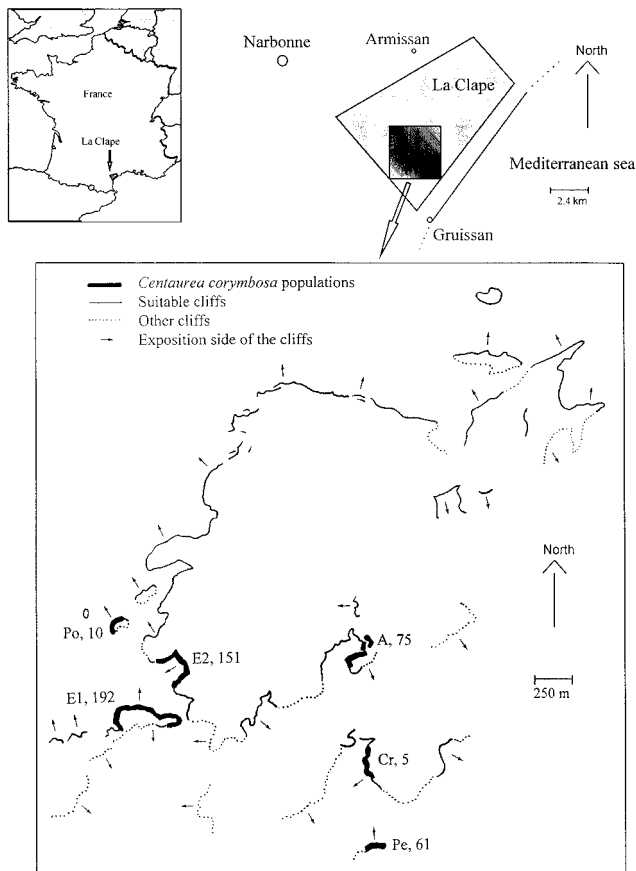‡To whom reprint requests should be addressed.

FIG. 1. Geographical location of the Massif de la Clape and distribution map of the *C. corymbosa* populations. Numbers after codes are numbers of flowering plants in 1995 (total size = 494).

colonization, possibly during the last glaciation. The species was first described in population A in 1788 (18). Populations E1 and E2 were found in 1852 (exicata from the general Herbarium of the Botanical Institute, Montpellier). Population Cr was described in 1892 (19). The first official reference to population Po is from 1983 only (20), but we suspect it could have been part of the population E1 and E2 when those were described in 1852. As for the last population, Pe, it had never been described before the present study.

Genetic Diversity and Population Genetic Structure. *Sampling technique and sample preparation.* Plant material was collected from a total of 221 individuals in all localities (see Table 1 for sample sizes per site). Young leaves were collected and kept frozen (−196°C) until processed. Samples were ground in the following extracting buffer: 0.1 M Tris·HCl (pH 7.6), 2 g of thioglycolic acid Na salt/50 ml, and 1 g of polyethylene glycol/50 ml.

Enzymatic systems. Out of 35 enzymatic systems tested on starch gels, 15 showed a good and repeatable activity (recipes available upon request to B.C.). From these systems, 19 loci could be recognized with clear electrophoretic patterns. Only 5 loci were found to be polymorphic. Genotypes were inferred directly from enzyme banding patterns based on knowledge of the overall conservation of isozyme subunits composition and isozyme number in plants (21).

Data scoring and data analysis. The percentage of polymorphic loci (P) and the genetic diversity [as measured by an unbiased estimate of H, the expected heterozygosity (22)] were calculated, both at the population and species levels, using BIOSYS package (23). To determine whether random mating occurred within populations, the conformation of genotype frequencies to Hardy–Weinberg expectations (F_{is}) was calculated and tested using GENEPOP (24, 25) for each population

and each locus. Population genetic structure was determined through comparisons of allele frequencies at polymorphic loci using Fisher's exact test (26), as calculated by GENEPOP. Pairwise F_{st}s between all pairs of populations and overall F_{st} were estimated using GENEPOP, which is based on Weir and Cockerham's method (27). Because only five loci were polymorphic, no attempt was made to calculate SD of F_{st} using, for example, resampling techniques. Using a computer program provided by J. Goudet (University of Lausanne), a Mantel test was performed between genetic and geographic distances among pairs of populations to determine a possible isolation by distance pattern of differentiation (28).

Seed Dispersal. Effective seed dispersal was estimated by counting and measuring seedling distances to the nearest reproducing plant. Seedlings were recorded in December 1995 within a 2-m radius around 42 isolated dead plants (16, 13, 7, 1, 2, and 3 plants from populations A, E1, E2, Cr, Po, and Pe, respectively) known to have flowered during the summer. Some seeds can remain viable over 1 year, so the flowering plants chosen were at least 5 m from any other plant flowering in either 1994 or 1995 so that they were very likely to be the maternal plants of the seedlings observed.

Additionally, to estimate the possibility of seed dispersal events at distances greater than 2 m, 13 microsites were surveyed for germinations in December 1995. They were variable in size (on average 2.8 m², ranging from 1 to 4.4 m²) and included no flowering plant in either 1994 or 1995. In 1995, one to 13 plants were flowering 2–10 m away from any given microsite. Therefore, the presence of seedlings in these quadrats in December 1995 would indicate the existence of seed dispersal from a source located at least 2 m away.

Pollen Limitation. Pollen availability was assessed through fertilization rates of mature capitula. In 1995, from the beginning (end of May) to the end (end of July) of the flowering period, 226 mature capitula were collected before seed dispersal on randomly chosen plants from A, E1, E2, and Pe. Approximately every 2 weeks, counts also were made of the number of flowering and senesced capitula in each population. From these data, and for each sampling date, overall flowering phenology of plants in each population was calculated as the number of senesced capitula divided by the total amount of senesced capitula at the end of the flowering period. Collected capitula were assigned to different flowering period classes: early, mid, and late flowering, according to cumulated proportions of senesced capitula in each population (<25%, between 25 and 75%, and >75%, respectively).

For each sampled plant, we recorded the distance to the nearest neighbor flowering plant (DIST) and the number of flowering plants in a 10-m radius (DENS). In each capitulum, we counted the number of disk florets (one ovule per floret) as well as the number of filled and aborted seeds. We then estimated the proportion of fertilized ovules per capitulum, taking into account only those capitula that were not obviously damaged by predators. A total of 206 capitula were included in the study (52, 49, 73, and 32 plants for populations A, E1, E2, and Pe, respectively).

Variation in fertilization rates was analyzed then for the effects of population, flowering phase, DIST, and DENS. The latter two variables were highly correlated (r = 0.64, P < 0.01, log-transformed data), so we tested them in separate analyses together with population and flowering period. We performed both ANCOVAs and ANOVAs. In the latter, levels for DIST and DENS corresponded to four different classes chosen so as to obtain approximately equal sample sizes (50 per class). ANOVAs [PROC general linear models (29)] were carried out on the angular transformed proportion of fertilized ovules. Because none of the interaction terms was significant, only main effects were included in the final model. Mean values of each class were compared using Duncan's Multiple Range test. Because both types of models, with either DIST and DENS as continuous covariates or categorical

variables, gave the same kind of results, only the latter will be shown.

Experimental Introduction. In November 1994, 1050 seeds of *C. corymbosa* were introduced to 21 microsites (≈ 1 m^2) on tops of cliffs located 0.5–2.5 km away from extant populations. Fifty seeds were individually placed in clefts in each microsite. Seed germination and seedling survival were recorded every 3 months over the 1st year. For comparisons with naturally established populations, 28 microsites (0.5–14 m^2) also were monitored for seedling survival in extant populations (A, Cr, E1, Pe, and Po). We compared survival rates over 1 year between introduced and natural populations for seedlings germinated in autumn 1994. Comparisons were carried out using the Mantel–Cox log–rank test for right censored data (30).

RESULTS

Genetic Diversity and Population Structure. All polymorphic loci were diallelic and fixed for one allele in two or three populations. Allelic frequencies for each polymorphic locus are given in Table 1. A total of 26.3% loci were polymorphic at the species level, but only between 10.5 and 15.8% were polymorphic at the population level (Table 1). Genetic diversity as measured by Nei's index (H) was 0.074 at the species level, ranging from 0.030 to 0.070 within populations. There was no relationship between the level of genetic diversity and the size of the population considered; both the smallest (Cr) and the largest (E1) populations showed similar values of genetic diversity (Table 1). Within any population and for any locus, the deviation of genotypic frequencies from Hardy–Weinberg expectations was not significantly different from 0 ($P = 0.09$ to 1; see Table 1 for average F_{is} per population), indicating no departure from random mating.

Overall F_{st} value was 0.35. This value, as well as each per locus F_{st}, was significantly different from 0 using Fisher's exact tests for overall structure ($P < 0.001$). Between pairs of populations, F_{st} ranged from 0.05 to 0.49. All pairwise comparisons carried out to test for differences in genetic structure between populations were significant (Fisher's exact test, $P < 0.05$). There was a significant positive correlation between genetic and geographic distances (Mantel test: r = 0.78, $P < 0.05$; Fig. 2).

Seed Dispersal and Pollen Availability. Three hundred and nine seedlings were found around the 42 flowering isolated plants. The seed dispersal curve obtained (Fig. 3) was highly leptokurtic. Mean dispersal distance was 32 cm. Although some seedlings were found to establish at more than 1 m away from the parent plant, 83% were found within a 50-cm radius of the plant. The furthest seedling was located at 168 cm. No seedling was found to establish in any of the 13 permanent microsites surveyed for germination even though several plants were flowering 2–10 m away.

There were 15–65 ovules (mean 43) per capitulum out of which, on average, 45% were fertilized. Both ANOVA models
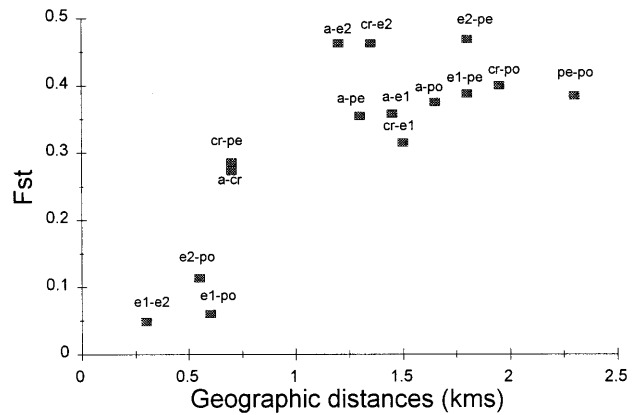

Fig. 2. Relationship between genetic (F_{st}) and geographic distances among *C. corymbosa* populations (Mantel–Cox test: r = 0.78; $P < 0.05$).

(either with DIST or DENS) used for testing pollen availability gave similar results for the effects of population and flowering period. There were highly significant differences among the three flowering periods ($P < 0.001$), with the maximum fertilization rate observed at midflowering (Table 2). Populations also differed, with population E2 showing a significantly ($P < 0.05$) larger fertilization rate (55.4%) than the populations E1 (41.2%), A (40.2%), and Pe (34.2%).

Mean distance (DIST) to the nearest flowering plant for all populations was 4.7 m, ranging from 0.05 to 150 m with a mode equal to 1 m. Mean density of flowering plants (DENS) in a 10-m radius was 6.7, ranging from 0 to 23. Mean fertilization rate of plants with reproducing individuals located less than 4 m was twice the one of plants for which the closest reproducing individuals was beyond 4 m (Table 2). One individual located 150 m from any flowering plant had a fertilization rate of only 9%. Similarly, mean fertilization rates progressively decreased from high densities (>9 flowering plants in a 10-m radius) to low densities (<2 flowering plants) (Table 2).

Experimental Introduction. In the experimental microsites, 259 seeds (24.7%) had germinated by December 1994. No seedling was found to establish outside the experimental quadrats where seeds were placed, indicating that seeds are not likely to have a high postdispersal movement when landing inside rock clefts. For quadrats surveyed in naturally established populations, a total of 175 germinations was recorded by the same date. There was no difference in seedling survival during the first year (December 1994-December 1995) between natural (9.8%) and introduced populations (11.3%) (Mantel–Cox log–rank test, $P = 0.82$) (Fig. 4). Thus, microsites are available for plant establishment in other localities.

Table 1. Sample sizes, allelic frequencies at polymorphic loci, percentage of polymophic loci, genetic diversity, and deviation to Hardy–Weinberg expectations at the population and the species level of *Centaurea corymbosa*

Populations	n	PGI a	DIA b	LAP b	CAT b	PER b	P	H	F_{is}
A	55	1	0.40	0.99	0.72	1	10.5	0.048	−0.04 NS
Cr	16	1	0.78	0.62	0.38	1	15.8	0.070	0.01 NS
E1	44	0.70	1	1	0.81	0.62	15.8	0.064	0.18 NS
E2	57	0.75	1	1	0.95	0.41	15.8	0.051	0.11 NS
Pe	32	1	0.70	0.41	1	1	10.5	0.048	−0.06 NS
Po	17	0.91	1	1	1	0.73	10.5	0.030	0.38 NS
All populations	221	0.87	0.79	0.89	0.83	0.75	26.3	0.074	0.07 NS
Fis		0.09 NS	−0.09 NS	−0.11 NS	0.12 NS	0.23 NS			
Fst		0.17 HS	0.43 HS	0.52 HS	0.23 HS	0.37 HS			
Fit		0.24	0.37	0.46	0.32	0.52			

All loci being diallelic, only the frequency of the most frequent allele is presented for each locus.
N, sample size; P, percentage of polymophic loci; H, genetic diversity; F_{is}, deviation to Hardy–Weinberg expectations; NS, not significant; HS, highly significant.
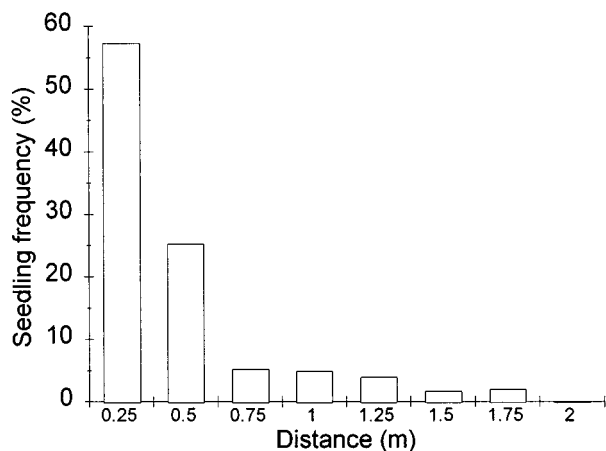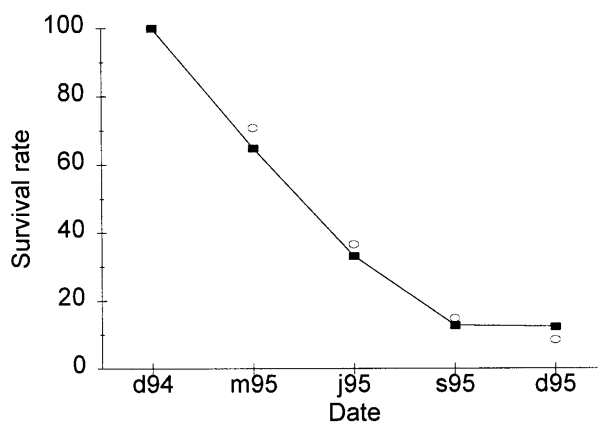
FIG. 3.   Distribution of seed dispersal distances from the maternal plant.

○ natural populations   ■ experimental populations

FIG. 4.   Seedling survival over 1 year in natural and experimental populations.

DISCUSSION

Levels and Distribution of Allozyme Variation. Overall genetic polymorphism in *C. corymbosa* is low compared with other endemic plant species (Table 3). Most of the endemic species referenced in Hamrick *et al.* (31) probably occupy a much larger area than that occupied by *C. corymbosa* (3 km^2). The amount of polymorphism at the species level in *C. corymbosa* is in fact similar to the average observed at the population level in endemic species (Table 3).

The strong differentiation among populations of *C. corymbosa* is very unusual. Indeed, although geographical range does not significantly correlate with the organization of allozyme variation in plants (31–34), life form and breeding system are both highly associated with differences in the proportion of the among-populations variation (31, 33). *C. corymbosa* exhibits a higher among-populations component of allozyme diversity than average perennial herbaceous or animal-pollinated species (Table 3). Recent studies of endemic, animal-pollinated species sharing with *C. corymbosa* both self-incompatibility and herbaceous perennial form have shown F_{st}s ranging from 7 to 15% (34–36). It is thus difficult to consider *C. corymbosa* as being made up of a single population, and one may infer from our results that, although spatial units are very close to one another (0.3–2.3 km), gene flow is highly restricted. The high correlation between F_{st} and geographical distance suggests a model of isolation by distance (28, 37), as found in several other studies on a broader geographical scale (38–40). Five of the six extant populations of *C. corymbosa* have been known to exist for a relatively long time (A, E1, E2, Cr, and Po have been known for 50–200 years), and no previously known population has disappeared (refs. 18–20; and various old *exicata* in the Herbarium of the Montpellier Botanical Institute). These historical data indicate the relative stability of the spatial distribution of the species. This stability is in contrast to what has been observed in another endangered species, the annual *Pedicularis furbishiae*, which exhibits exceptionally large local extinction rates (41). This pattern of population dynamics might explain that polymorphism was lacking in *P. furbishiae* populations, subjected to frequent bottlenecks, whereas we found substantial polymorphism in *C. corymbosa* populations.

Seed Dispersal Study. As commonly observed for most species (42, 43), the seed dispersal distance curve of *C. corymbosa* is leptokurtic. Dispersal distances in *C. corymbosa* are strikingly low (mean 32 cm, maximum 168 cm) compared with other Asteraceae (44, 45) but are similar to other *Centaurea* species like *C. solstitialis* (46, 47). The leptokurtic shape of the dispersal curve does not preclude the possibility of long distance dispersal (48). Indeed, some authors (44, 45) have pointed out that, often, in other species of Asteraceae, a number of plumed seeds were actually lifted up by wind and transported to far distances that could not be taken into account in their studies. This, however, has never been observed on *C. corymbosa*, even with wind speeds above 100 km/h, which are frequent at the Massif de la Clape. Dispersal distances of *C. corymbosa* seeds thus more closely resemble those observed after ballistic dispersal (49, 50) than after wind dispersal. Although all seeds have a pappus approximately as long as the achene, it does not seem to help seed disperse by wind beyond nearby microsites. We believe it functions as a parachute-like structure that facilitates (*i*) seed extraction from the capitulum when it opens and (*ii*) keeping the embryo forward when the wind pushes the seed on rocks before landing into holes with the embryo at the bottom of the cleft, a position favorable for germination.

Seeds of *C. corymbosa* also are adapted to dispersal by ants; they have a true, well developed elaiosome as in other *Centaurea* species (51). Dispersal by ants (i.e., *Crematogaster scutellaris*) occasionally has been observed in the field but probably occurs over short distances as is generally the case for this dispersal mode (42, 52–54). Dispersal by adhesion may occur over much longer distances than dispersal by ants (55, 56) but is very difficult to observe (56, 57). The barby and sticky bristles in the pappus indicate possible dispersal by adhesion to fur or feathers of vertebrates (56, 57). We have no clear evidence of this in *C. corymbosa* although it could occur on the feathers of *Bubo bubo*, a nocturnal raptorial bird sharing the same habitat. Indeed, some individuals of this great owl are living in sites E1, E2, and Po, and the microsites they visit (as evidenced from owl pellets) are also *C. corymbosa* microsites. The fact that microsites only a few meters away from flowering

Table 2.   Fertilization rate of capitula in relation to flowering period, distance, and density class

	Flowering period			Distance class, m				Density class			
	Mid	Early	Late	0-0.3	0.3-1	1-4.1	>4.1	>9	5-9	2-5	0-2
Sample size	85	74	47	52	52	52	50	44	57	49	56
Fertilization rate, %	55.6a	42.4b	29.4c	54.5a	50.4a	45.0a	29.0b	56.8a	49.7ab	41.2bc	33.8c

For each variable and each effect, numbers followed by different letters are significantly different (Duncan's Multiple Range test, $P < 0.05$). Numbers followed by at least one common letter are not significantly different.

Table 3.　Comparison between levels and distribution of allozyme variation found for *C. corymbosa* and mean values for various categories of plant species reviewed by Hamrick and Godt (31)

| Categories | Levels of allozyme variation | | | | Distribution of allozyme variation among populations |
| | At the species level | | At the population level | | |
	P	H	P	H	
C. corymbosa	26.3	0.074	13.1	0.052	0.35
Dicots	44.8	0.136	29.0	0.096	0.27
Short-lived perennials	41.3	0.116	28.0	0.096	0.23
Endemic	40.0	0.096	26.3	0.063	0.25
Outcrossing animal	50.1	0.167	35.9	0.124	0.20

P, percentage of polymophic loci; H, genetic diversity.

plants were not colonized suggests, however, that dispersal by more than 2 m is likely to be a rare event.

Pollen Availability and Dispersal. The data on ovule fertilization rates clearly demonstrate that pollen limitation occurs in *C. corymbosa*. Because of self-incompatibility, pollination of capitula depends on neighboring flowering plants. The fertilization rate was larger when the largest number of plants were flowering in any given population (midflowering period vs. early and late), and the fertilization rate was low for those plants that were in an area of low density of flowering plants. These results suggest that pollen and/or pollinator availability is restricted in *C. corymbosa*, as in *Dianthus deltoides* in fragmented areas (58).

The fertilization rate also was low for plants that were more than 4 m away from the nearest neighboring flowering plant. This is consistent with other studies showing that most of the pollen is carried over very short distances by insects in animal-pollinated plants (50, 59–63). We would then expect to find small neighborhood areas (60, 64) within which most of the pollen is dispersed. In fact, no departure from panmixia was found for any of the *Centaurea* populations. Such a discrepancy between direct estimates of pollen movement and indirect estimates of gene flow by paternity analysis (65) or by genetic distances (66) also has been observed in *Ipomopsis aggregata*, another self-incompatible, animal-pollinated plant. This may be due to both postpollination events [i.e., preferential fertilization with respect to distance, (67)] and undetected, rare long distance gene flow (68). Such events would be sufficient to prevent genetic differentiation among neighborhoods within populations (68, 69).

Out of 9 capitula from 6 flowering plants that were 30–150 m from their nearest flowering neighbor, all produced some, however few, seeds. No seed was obtained after artificial self-pollination of 50 capitula (24 plants) in the A, E1, and Pe populations (17), so we assume that the 6 isolated plants were visited at least once by pollinators, indicating that relatively long distance pollination may occur in *C. corymbosa*. The strong genetic differentiation that was observed among populations suggests, however, that such long distance pollination events are very rare among populations.

Experimental Introduction Study. Although there is observational evidence that the geographic distribution of many species is limited by their colonization ability (2, 70), few experimental studies have validated this claim. For example, Levin and Clay (71) demonstrated that *Phlox drummondii* can successfully grow and reproduce at the species margin. Other authors have emphasized the importance of appropriate management practices that insure the viability of newly created populations of *Amsinckia grandiflora* (72), *Erysimum capitatum*, and *Oenothera deltoides* (73). Finally, Primack and Miao (74) concluded from their experimental study on four annual plant species that both site suitability and dispersal ability were limiting local distribution. Most experimental studies of this kind only consider establishment in new sites without demographic comparison with naturally

occurring populations. An exception to this is the study by Prince and Carter (75), which showed similar performances of *Lactuca serriola* in natural and experimental sites well beyond its natural limits, further verifying the importance of lack of dispersal as a major component of plant distribution patterns.

To demonstrate the successful establishment of a new population would need monitoring over several generations (possibly 10–15 years for *C. corymbosa*). Our comparison of survival rates of seedlings in natural and artificially introduced populations nevertheless suggests that the new sites are suitable because the early stages are the most critical of the life cycle in plants (76). Thus, if seeds had been able to arrive by themselves in these neighboring sites (0.5–2.5 km), a population might have been able to establish. Germination rate times seedling survival is very low ($\approx$3% for the experimental introduction, a figure likely to be even lower in naturally occurring populations because a number of seeds do not actually land in rock clefts), so long distance colonization events are very unlikely to occur.

CONCLUSION

Both indirect and direct measures indicate that gene flow in *C. corymbosa* probably occurs mainly by pollen, within populations. It is very restricted among populations and primarily occurs between adjacent ones (given the isolation by distance pattern of differentiation). In the fragmented rocky habitat of the Massif de la Clape, populations are well delimited by the cliffs and are surrounded by areas of either pinewood or garrigues with dense bushes. The absence of plant outliers (77) in these areas probably decreases the possibility of pollen flow between populations.

One might ask whether limited seed dispersal results from natural selection against dispersal, as has been suggested to explain the lack of long distance dispersal mechanisms in desert plant species (78). Indeed, according to several theoretical studies (4, 5, 79, 80), unsuitable patches around populations select against dispersal. Therefore, the habitat structure would have two effects: a direct one, whereby dispersal is prevented among suitable sites, and an indirect one, whereby eventual mutant genotypes with a larger dispersal ability would be selected against. We now suggest reasons why the second effect also is likely to occur.

In addition to low dispersal ability, the colonization ability of *C. corymbosa* is likely to be further lowered by monocarpy and self-incompatibility. Hence, if, by chance, some seeds are dispersed a long way (i.e., several hundreds of meters to several kilometers) to a suitable site, germinate, and grow into adult plants, they are likely to die without producing any offspring if they do not flower simultaneously. The reason why neither self-compatibility nor iteroparity have evolved in *C. corymbosa* is puzzling. Although there could be a purely genetic constraint answer, the presence of self-compatibility and iteroparity elsewhere in the family suggests against this possibility. Furthermore, there are numerous examples in which self-incompatibility has been lost (81) or in which both self-incompatible and self-compatible genotypes coexist (82). Some rare plant species appear to be able to break self-incompatibility, especially those subject to frequent population bottlenecks. In the self-incompatible species *Aster furcatus* for instance, it has been found recently that some genotypes (4–6 out of 22 tested) were fully or partially self-compatible (82). Out of 50 capitula (24 plants) of *C. corymbosa*, none set seeds after selfing (17). The reason why self-compatibility has not evolved in this species could be inbreeding depression: in an outcrossing species, inbreeding depression due to either heterozygote advantage or recessive deleterious alleles at many loci is expected to be strong, so that any mutant genotypes with a large selfing rate (e.g., a self-compatible genotype) would produce a progeny of low fitness. *C. corymbosa* population sizes are very small, so one might expect either a purging or a fixation of deleterious alleles to have occurred and thus a decrease of inbreeding depression at the population level (83, 84). However, recent studies on self-compatible, outcrossing *Scabiosa* species (85) have shown that inbreeding depres-

sion may persist even in small populations. It could be that inbreeding depression in *C. corymbosa* is still so strong that any mutant appearing in a population would be strongly selected against. To select for a self-compatibility allele, the mutation would then have to occur when a new population is being established (founder event). Mutations are more likely to occur in large populations than in small ones, so there is a double catch-22 problem here: To select for self-compatibility, one needs either a small population or a newly founded population. To found a new population, one needs self-compatibility. To have self-compatibility appearing, one needs a large population. Only when bottlenecks frequently occur, as seems to be the case for some species (82), can we expect mutation toward self-compatibility to both occur and be selected for. It could be that such founder events are too rare in *C. corymbosa*, which has become trapped in its own deleterious system.

Two implications of this study can be advanced for the future of *C. corymbosa*. First, because extant populations are very small and quite isolated despite their proximity, they are subjected to genetic drift and mutational meltdown (86). Hence, they incur the loss of genetic variation, generally thought to be essential for long term survival (87–92) and the fixation of deleterious alleles (12, 86, 93). Second, because of the lack of colonization ability, the species is dependent on the persistence of existing populations, themselves at the mercy of stochastic environmental fluctuations (41, 94, 95), which can be very high in Mediterranean regions. One could argue that the species is still there and might look perfectly well adapted to the rocky habitat of the Massif. We have, however, some demographic evidence (unpublished data) that overall population size is decreasing from year to year, suggesting that some management decisions will have to be made if one is to prevent this species from becoming extinct. Such managements would include experimental introductions from a few individuals (to create several founder effects) into new sites and opening of corridors between them (to increase selection for long distance dispersal). The demographic and genetic survey of these experimental populations would also bring new insights into evolutionary mechanisms such as the role of initial genetic diversity and population size.

We predict that the type of biological situation discussed in this study could well be applied to many rare species sharing common life histories with *C. corymbosa*. Moreover, the experimental framework considered, whereby it is possible to suggest ongoing mechanisms for species extinction, seems ideal for testing theoretical ideas about adaptation, genetic and demographic constraints, and conservation.

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